View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Kidney International, Vol. 57 (2000), pp. 2099–2106

DIALYSIS – TRANSPLANTATION

Impaired monocyte CD11b expression in interstitial inflammation in hemodialysis patients

Pia Thylén, Joachim Lundahl, Eva Fernvik, Reidar Grönneberg, Gunilla Halldén, and Stefan H. Jacobson

Department of Nephrology and Department of Clinical Immunology, Karolinska Hospital and Karolinska Institute; and Division for Respiratory and Allergic Diseases, Department of Medicine, Huddinge Hospital and Karolinska Institute, Stockholm, Sweden

Impaired monocyte CD11b expression in interstitial inflammation in hemodialysis patients.

Background. It is not known to what extent intravascular phenotypic alterations in adhesion molecule expression induced by hemodialysis influence the recruitment of monocytes and their ability to up-regulate CD11b at the local site of inflammation in the interstitium. Using a skin suction chamber technique, we addressed these issues in eight hemodialysis patients and in eight healthy subjects.

Methods. Two skin blisters were raised on the forearm of each individual and blister exudate collected. The blisters were then stimulated with autologous serum (active blister, intense inflammation) or buffer (control blister, intermediate inflammation), respectively. Thereafter the patients were treated with Cuprophan hemodialysis for four hours. After 10 hours, the exudate was aspirated from each chamber in all subjects. Monocyte count and expression of CD11b were analyzed in serum and blister fluid by flow cytometry. Then, monocytes from healthy blood donors were incubated in blister fluid from patients and healthy subjects in order to determine the local chemotactic activity in terms of CD11b up-regulation. Monocyte chemotactic protein-1 (MCP-1), a marker of systemic monocyte chemotactic activity, was also analyzed in serum at 0 and 10 hours in all individuals.

Results. The number of monocytes at the site of inflammation in the interstitium in hemodialysis patients correlated with the expression of CD11b on transmigrated cells ($r = 0.78$, $P < 0.001$). Monocytes collected in the active blister fluid of dialysis patients expressed equal levels of CD11b as cells collected from healthy subjects. By contrast, monocytes collected from the control blisters of patients expressed lower levels of CD11b than cells from healthy subjects ($P < 0.01$), despite equal interstitial biological activity of CD11b-mobilizing factors in blister fluid from patients and healthy subjects and the fact that patients had higher systemic chemotactic activity in terms of MCP-1 concentration in serum ($P < 0.001$).

Conclusion. Monocytes from hemodialysis patients have the capacity to mobilize CD11b to the same extent as cells from healthy individuals at the inflammatory spot, but more intense stimuli are required for such actions, probably because of a transient refractoriness.

Key words: CD11b/CD18, adhesion molecules, monocyte chemoattractant protein-1, skin suction chamber, transmigration.

Received for publication May 14, 1999
and in revised form November 10, 1999
Accepted for publication November 24, 1999

© 2000 by the International Society of Nephrology

The migration of leukocytes from the peripheral circulation into interstitial spaces involves sequential interactions between activation-dependent molecules on the endothelium and leukocytes [1]. The initial recognition and adhesion of the circulating leukocyte to vascular endothelium at the site of inflammation are mediated by selectins. The second phase is mediated through integrin molecules and involves activation of the leukocyte resulting in firm adhesion to the endothelium. The adherent leukocytes then diapedese between endothelial cells and migrate through extravascular tissue along gradients of chemoattractants diffusing from the inflammatory site [2]. When the specific leukocyte has reached the point of highest chemokine concentration, it arrests and secretes cytokines and additional chemokines, resulting in further leukocyte accumulation. Neutrophils mediate an early response to infection or tissue injury, whereas monocytes and lymphocytes typically are recruited in the later stages of the inflammatory response. It has been proposed that several chemokines are involved in leukocyte recruitment but also that certain chemokines, for example, monocyte chemotactic protein-1 (MCP-1) and interleukin-8 (IL-8), may show restricted tissue expression and as a result are involved in organ-specific recruitment [1]. MCP-1 is a monocyte specific chemoattractant, and IL-8 acts as a neutrophil chemotaxin [2, 3]. Little is known about the factors that control the type of chemokine generated and what eventually terminates chemokine and cytokine production, which down-regulates the inflammatory response.

Hemodialysis results in profound activation of the alternative pathway of the complement system, which is associated with transient leukopenia and followed by re-

bound leukocytosis [4]. This unphysiological activation of monocytes and neutrophils, resulting in rapid alterations in adhesion molecule phenotypes, occurs in the extracorporeal circuit far away from the respective ligand on the endothelial cell lining [5–7]. These phenotypic changes result in defective adhesion of inflammatory cells to human endothelial cells [5, 7]. It is less well known to which extent these alterations in adhesion molecule expression and cell count influence the capacity of monocytes and neutrophils to be recruited into inflammatory sites.

In an attempt to address this issue, using a skin suction chamber technique, we examined the in vivo ability of monocytes to accumulate at induced inflammatory foci in patients on hemodialysis.

METHODS

Cell count and expression of adhesion molecule CD11b/CD18 were measured at 0 hours and after 10 hours on monocytes in the peripheral circulation and in skin blister fluid collected from hemodialysis patients on the day of dialysis and from healthy subjects.

Study population

Eight hemodialysis patients (mean age 49 ± 7 years, range 35 to 67 years) undergoing maintenance hemodialysis with polysulfone dialyzers (F6 HPS and F7 HPS; Fresenius AG, Bad Homburg, Germany) three times a week participated in the study. All patients had been on hemodialysis for more than six months. The dialyses in the study were part of the routine dialysis program. All patients suffering from infectious diseases, diabetes mellitus, chronic lung disease, or inflammatory diseases, as well as patients receiving antibiotics, corticosteroids, or nonsteroidal anti-inflammatory agents were excluded. Informed consent was obtained, and the study was approved by the Ethics Committee of the Karolinska Hospital. Eight healthy subjects (mean age 35 ± 2 years, range 24 to 52 years) without any medication were also examined.

Hemodialysis

All patients were switched to dialysis with Cuprophan hollow fiber dialyzers with a membrane area of 1.8 m^2 (Gambro Dialysatoren GmbH & Co., Hechingin, Germany) for four weeks before entering the study. Our hypothesis was that a more profound disturbance in adhesion molecule expression, which occurs during Cuprophan hemodialysis [6–8], would influence cell recruitment and expression of CD11b/CD18 at the local site of inflammation in the interstitium. Dialyzers were not reused. On the day of investigation, hemodialysis was performed for four hours with the Cuprophan dialyzer and a blood flow of 300 mL/min. Heparin doses were individualized and given as an initial bolus dose followed by a continuous intravenous infusion until one hour before termination of treatment.

Blood samples were collected prior to dialysis and 10 hours after start of dialysis in ethylenediaminetetraacetic acid (EDTA) tubes, and kept on ice to prevent further complement activation and receptor modulation. Blood from healthy subjects was collected at the corresponding time points.

Skin suction chambers

The monocyte response to cutaneous inflammation was assessed by the skin suction chamber technique in both patients and healthy subjects. Two skin blisters were raised on the volar surface of one of the forearms (in hemodialysis patients the blood access-free forearm was chosen; Fig. 1). After disinfection, the suction chamber was placed on the shaved, cleaned skin and secured on the arm. A constant vacuum (300 mm Hg) was applied by continuous gentle suction (Electronic Diversities, Finksburg, MD, USA). Blister formation was promoted by heating (39°C) until the blisters were sufficiently developed. Two 9 mm diameter blisters were formed in two to three hours. The vacuum was then released, and the suction chamber was removed. The blisters were covered overnight with a plastic eye chamber (Augenverband S; Lohmann GmbH, Munich, Germany). The next morning, 12 to 14 hours after the formation of blisters, the roof of the blisters was carefully removed after the blister fluid had been aspirated, pooled, and saved for further analysis. This pool of exudate was designated 0 hours and, in the group of patients, represented the conditions in the uremic state. To protect the intact skin from irritation, a transparent sterile adhesive plastic film (Tegaderm; 3 M Pharmaceuticals, Loughborough, UK) with a 10 mm diameter hole was applied around the exposed blister floor. Skin bond cement (Smith & Nephew United, Inc., Largo, FL, USA) was then applied around the bottom edge of sterilized open-bottom plastic skin chambers (volume 1 mL), which were placed over the unroofed blisters and secured. In the proximal chamber, designated the active chamber, 1 mL of heparinized autologous serum was added (6 μL heparin added to 1 mL serum). In the distal chamber, designated the control chamber, 1 mL phosphate-buffered saline (PBS) and 6 μL heparin were inserted. The administration of serum and buffer in the respective chambers was done in order to induce a difference in intensity of inflammation in the blisters: an intense inflammation in the chamber with serum and an intermediate in that containing buffer. The autologous serum was collected the day before, centrifuged for 15 minutes at 4°C, and immediately frozen at −70°C. During the first four hours of incubation, Cuprophan hemodialysis treatment was given to the patients. After 10 hours of incubation, the fluid was aspirated from each chamber and placed on ice (Fig. 1). The blister

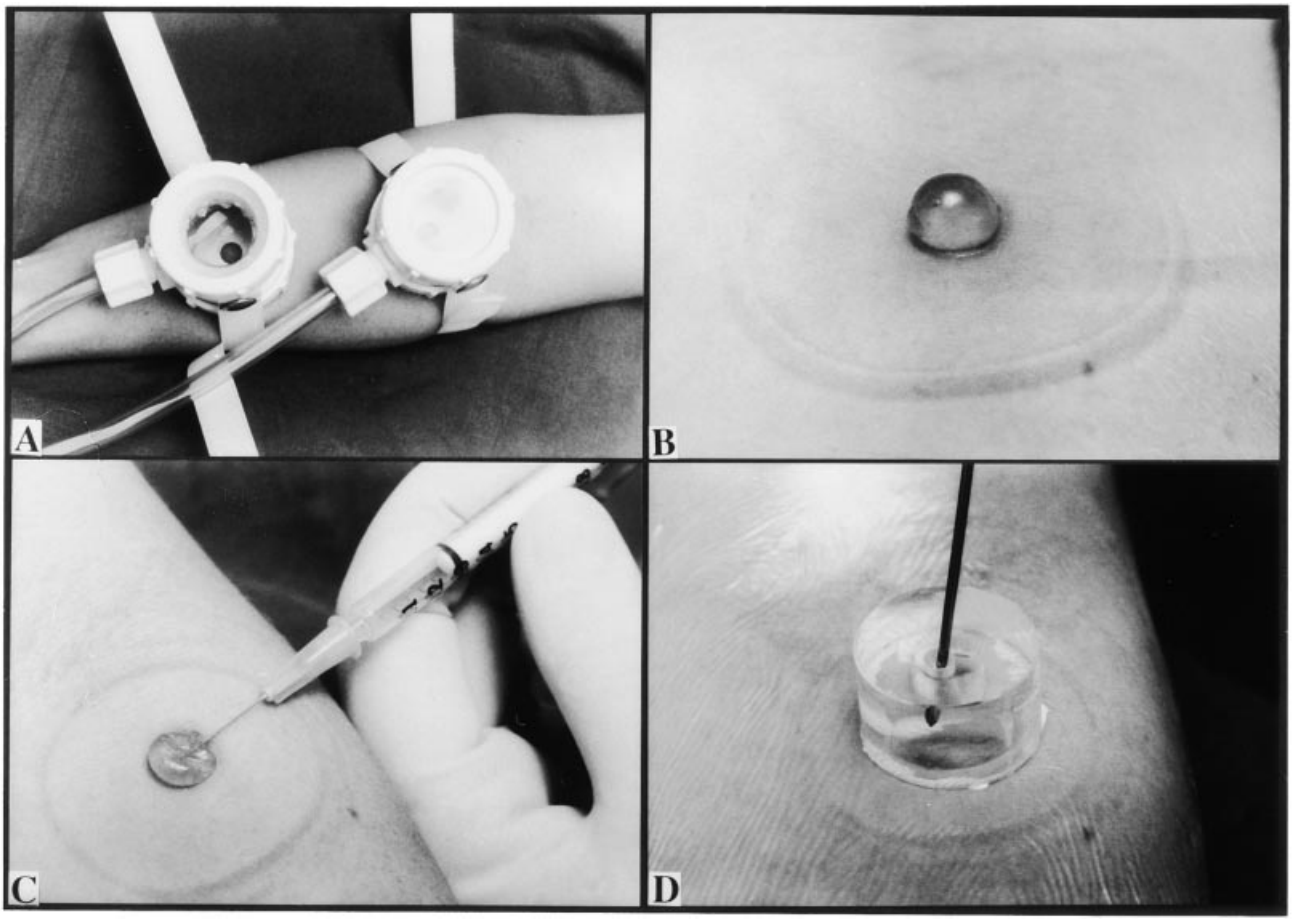

Fig. 1. Formation of blisters. (*A*) A suction chamber was placed and secured on one of the forearms, and a constant vacuum was applied. (*B*) Two 9 mm blisters were formed in two to three hours. (*C*) The next morning, 12 to 14 hours after blister formation, blister fluid was aspirated. (*D*) The roof of the blisters was carefully removed, and a sterilized open-bottom plastic skin chamber was placed over the unroofed blisters and was secured. Thereafter, buffer or serum was added, as described in the **Methods** section.

fluid was centrifuged at 300 × *g* for five minutes at 4°C, and the pellets were resuspended in PBS-EDTA and used for flow cytometric analysis of monocytes. The rationale for choosing 10 hours of incubation was based on a previous study in which we showed that phenotypic alterations in adhesion molecule expression on monocytes persist up to 10 hours after Cuprophan hemodialysis [8].

Preparation of peripheral leukocytes

Ethylenediaminetetraacetic acid blood was hemolyzed in 100 μL portions by dilution in 2 mL 4°C isotonic NH_4Cl-EDTA "lyzing solution." After five minutes of incubation at 4°C, the cells were centrifuged at 300 × *g* for five minutes at 4°C. The leukocytes were washed once and resuspended in 100 μL cold 0.15 mol/L phosphate buffered saline (PBS) supplemented with 0.1 mmol/L EDTA and 0.2% Na-azide (PBS-EDTA) before immunostaining.

Number of monocytes and analysis of CD11b/CD18 expression on cells in skin blister exudate and in the peripheral circulation

In the blister exudate (one pooled sample aspirated from overnight intact blisters and two samples drawn after 10 h from the active and control chambers, respectively), monocytes were counted using a flow cytometer (Epics Elite; Coulter Inc., Hialeah, FL, USA). This instrument gives the actual number of cells and the mean fluorescence intensity (MFI), which represents the density of the antigens of the cell population within a chosen field. CD11b/CD18 expression on monocytes was analyzed by adding 5 μL of phycoerythrin-conjugated monoclonal anti-CD11b (Dako AS, Glostrup, Denmark). An appropriate concentration of an isotype-matched control antibody was used to define the cut-off for positive fluorescence, which was the 99th percentile of the distribution of the cells labeled with the control antibody, PE-conjugated $IgG2_a$. Cell count and CD11b expression on

monocytes were also analyzed in peripheral blood in samples obtained at both time points.

In vitro mobilization of CD11b on monocytes from healthy blood donors following incubation in blister fluid from patients and healthy subjects

Monocyte preparations from healthy blood donors were incubated in vitro in 100 μL cell-free skin blister fluid obtained from 0 and 10 hours of active blister (stimulated with autologous serum, intense inflammation) and control blister (stimulated with PBS buffer, intermediate inflammation). Preparations were diluted 1/2 in 1640 RPMI medium, supplemented with 0.01 mol/L HEPES and 10% heat-inactivated fetal calf serum (RPMI-HEPES; GIBCO Ltd., Paisley, Renfrewshire, UK) and were incubated at 37°C for 15 minutes. Monocytes activated in the presence of 5×10^{-7} mol/L formyl-methionyl-leucyl-phenylalanine (fMLP; Sigma Chemical Co., Dorsett, UK) for 15 minutes at 37°C, served as a positive control, and monocytes incubated in RPMI-HEPES alone for 15 minutes at 37°C and 4°C, respectively, served as negative controls. After incubation, the cell suspensions were washed once in PBS-EDTA ($300 \times g$ for 5 min, 4°C) and resuspended in 100 μL PBS-EDTA and kept at 4°C until immunostaining and flow cytometric analysis (discussed previously in this article).

Analyses of MCP-1

Monocyte chemotactic protein-1 levels in serum were measured with a commercially available immunoassay (the Quantikine Human MCP-1 Immunoassay; R&D Systems, Inc., Minneapolis, MN, USA). The assay was done according to the manufacturer's instructions, and the minimum detectable concentration of MCP-1 was 10 ng/L.

Statistical analysis

Results are expressed as mean ± SEM. The figures are presented as boxplots showing the 10th, 25th, 50th (median), 75th, and 90th percentiles of a variable. Statistical comparisons were made using analysis of variance (ANOVA), Mann Whitney *U*-test, and the Wilcoxon signed-rank test for paired and unpaired observations.

RESULTS

Number of transmigrating monocytes into skin blister exudate

The number of monocytes collected in skin blister exudate from dialysis patients in the uremic state (before dialysis) and healthy subjects at 0 hours and in the control chamber (stimulated with buffer) and active chamber (stimulated with human serum) at 10 hours is presented in Figure 2. The number of monocytes in the active chamber (intense inflammation) at 10 hours was significantly higher as compared with in the control chamber (intermediate inflammation) in both patients and healthy subjects (Fig. 2). There was no significant difference between the number of monocytes in skin blister fluid collected from patients as compared with healthy subjects (Fig. 2).

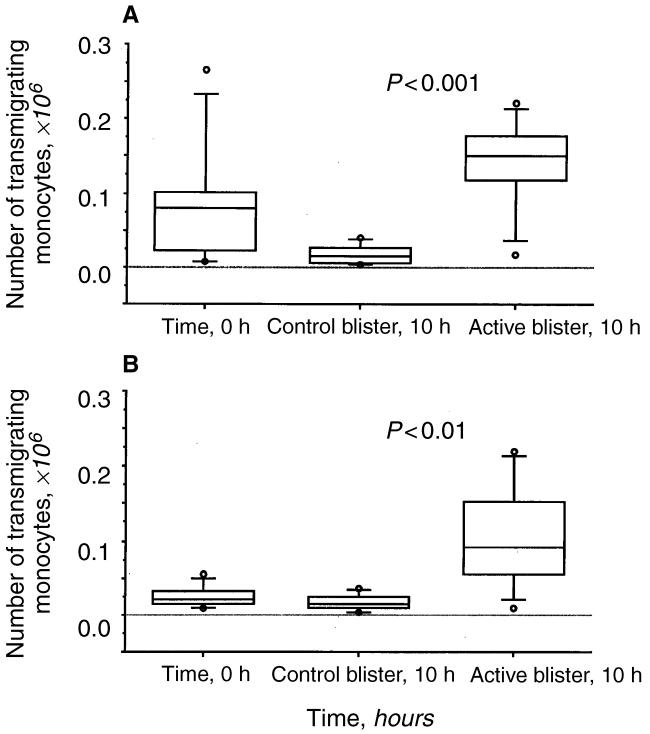

Fig. 2. Number of transmigrating monocytes into skin blisters of (*A*) hemodialysis patients and (*B*) healthy subjects. This shows the 10th, 25th, 50th (median), 75th, and 90th percentiles of a variable. $P < 0.001$ in patients (A) and $P < 0.01$ in healthy subjects (B) for control blisters vs. active blisters.

Expression of CD11b/CD18 on monocytes in the peripheral circulation

The expression of CD11b/CD18 on monocytes in the peripheral circulation was similar in patients and healthy subjects at both 0 and 10 hours. After 10 hours, 6 hours after termination of Cuprophan hemodialysis, the expression of CD11b on the population of monocytes present in the peripheral circulation was higher as compared with before dialysis ($P < 0.05$; Fig. 3).

Expression of CD11b/CD18 on transmigrating monocytes in skin blister exudate

The expression of CD11b/CD18 on transmigrating monocytes was significantly higher in the skin blister fluid at different time points as compared with in the peripheral circulation at the corresponding time points

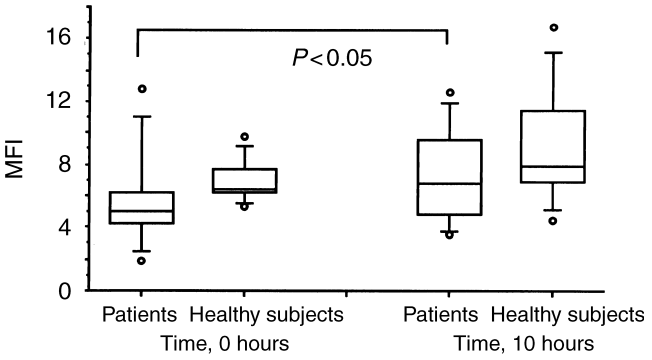

Fig. 3. Expression of CD11b/CD18 on monocytes in the peripheral circulation of hemodialysis patients and healthy subjects. This shows the 10th, 25th, 50th (median), 75th, and 90th percentiles of a variable. $P < 0.05$ in patients for time 0 vs. time 10 hours.

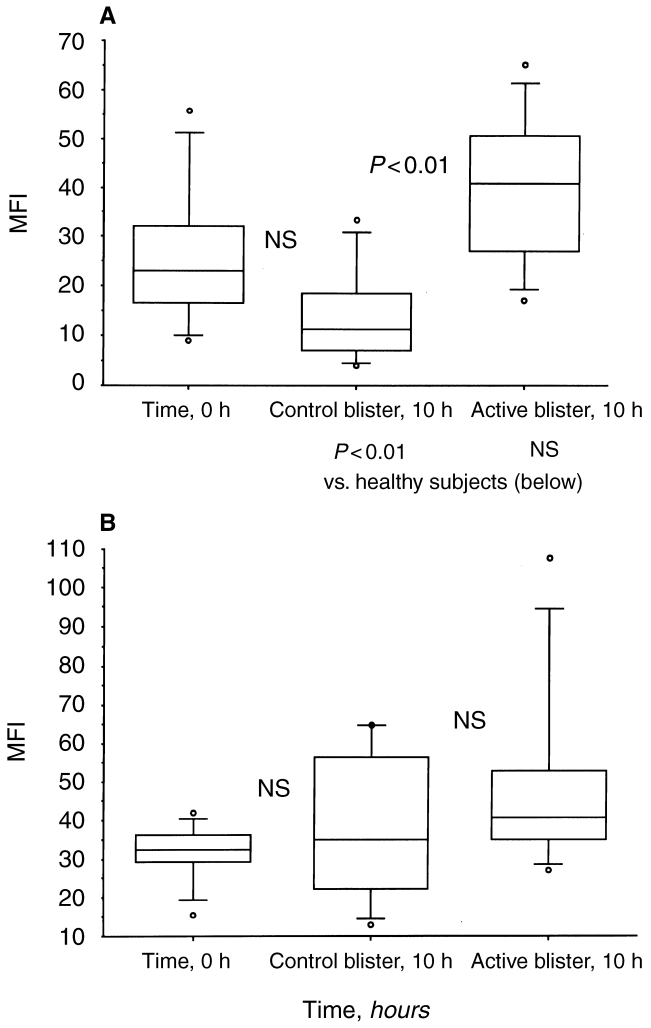

Fig. 4. Expression of CD11b/CD18 on transmigrating monocytes in skin blisters of hemodialysis patients (discussed previously; *A*) and healthy subjects (discussed later in this article; *B*). This shows the 10th, 25th, 50th (median), 75th, and 90th percentiles of a variable. Please note the differences in scales in the two panels. In patients (A), $P < 0.01$ control vs. active blisters; all other comparisons were not significant.

($P < 0.001$ for all of the comparisons; Figs. 3 and 4). The expression of CD11b/CD18 on transmigrating monocytes from patients on hemodialysis was significantly higher in the active chamber (containing autologous serum, intense inflammation) as compared with in the control chamber (containing buffer, intermediate inflammation) at 10 hours ($P < 0.01$; Fig. 4). There was, however, no significant difference in the expression of CD11b/CD18 on transmigrating monocytes collected from the corresponding skin blisters of healthy subjects (Fig. 4). The expression of CD11b/CD18 on transmigrating monocytes was significantly higher in the control chamber of healthy subjects as compared with in the corresponding chamber of hemodialysis patients ($P < 0.01$; Fig. 4).

The number of monocytes at the site of inflammation in the interstitium in hemodialysis patients (pooled data from all three blisters) correlated with the expression of CD11b on transmigrated cells ($r = 0.78$, $P < 0.001$; Fig. 5).

Incubation of monocytes from healthy donors in skin blister fluid

Monocytes from healthy blood donors were incubated in the three pools of skin blister fluid (time 0, control and active chambers, altogether 50 experiments) from patients and healthy subjects in order to determine the local biological activity of CD11b-mobilizing factors. There were no significant differences in CD11b expression on monocytes, incubated in the respective pool of skin blister fluid, between patients and healthy subjects (0 h, 16.6 ± 1.9 MFI vs. 15.7 ± 0.7 MFI; control chamber, 18.2 ± 1.7 MFI vs. 15.8 ± 1.2 MFI; active chamber, 21.4 ± 1.2 MFI vs. 22.2 ± 1.0 MFI). In both patients and healthy subjects, the CD11b expression was higher on cells incubated in fluid from the active chamber as compared with the cells incubated in control chamber fluid (patients, $P < 0.05$; healthy subjects, $P < 0.001$). Thus, the biological activity of CD11b-mobilizing factors at the local site of inflammation in the interstitium was equal in patients and healthy subjects.

Concentration of MCP-1 in serum

The systemic concentration of MCP-1 was higher in patients both before hemodialysis (239 ± 20 ng/L) and at 10 hours (202 ± 14 ng/L) as compared with in healthy subjects at the corresponding time points (73 ± 10 and 50 ± 7 ng/L, $P < 0.001$ for both comparisons). There were no significant differences between values at 0 hours compared with 10 hours in patients or healthy controls. The serum concentration of MCP-1 at 0 hours correlated with the number of transmigrating monocytes when all individuals were evaluated together ($r = 0.65$, $P < 0.01$), but not in patients or healthy subjects separately.

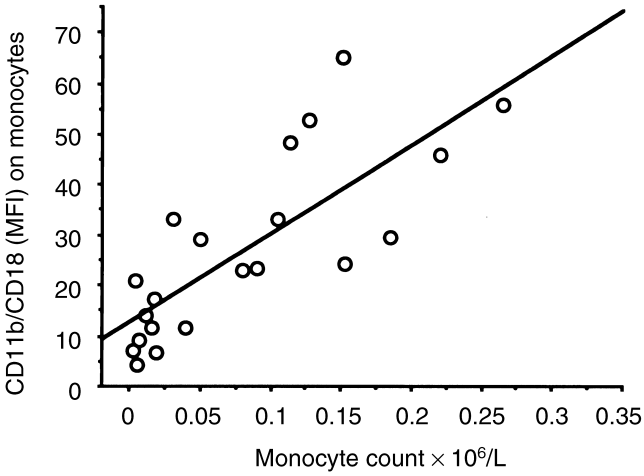

Fig. 5. Correlation between the number of monocytes at the site of inflammation in the interstitium in hemodialysis patients and the expression of CD11b/CD18 on transmigrated cells ($r = 0.78$, $P < 0.001$).

DISCUSSION

In this article, we show that the number of transmigrating monocytes to inflammatory foci in the interstitium is similar in patients and healthy subjects. However, monocytes from hemodialysis patients, recruited to an intermediate inflammatory site, had mobilized CD11b to a lesser extent as compared with monocytes from healthy subjects. This was not due to a lower biological activity of chemotactic factors in skin blister fluid and in peripheral blood from patients. In fact, blister fluid from patients contained an equal activity of CD11b-mobilizing factors as compared with healthy subjects, and serum contained a higher concentration of MCP-1.

This is the first investigation of hemodialysis patients in which the ability of monocytes to transmigrate and to mobilize adhesion molecules has been examined in the interstitial fluid at the local site of inflammation. In previous studies, these issues have been addressed and examined in a number of clinical and experimental models in which different cell populations collected before, during, and after hemodialysis have been studied [5–10]. One major problem in previous examinations is that it is very difficult to clearly define the population of monocytes studied, since cells are sequestrated in the microcirculation during the first part of dialysis and other cell populations mobilized later during dialysis resulting in rebound leukocytosis, which especially occurs in dialysis treatments using complement-activating membranes such as Cuprophan [5–12].

To address the question of whether the previously described hemodialysis-induced effects on circulating monocytes during and after hemodialysis affect their ability to accumulate at the inflammatory site, we applied a blister chamber technique. Two skin blisters were induced, representing three different states of inflammation. Cells collected by aspiration at 0 hours, 12 to 14 hours after the formation of blisters, before the administration of either buffer or autologous serum, represent the uremic state. It must be emphasized that no hemodialysis was performed during this period as opposed to the following blister settings. An intermediate inflammatory reaction was induced by removal of the roof of one of the blisters followed by instillation of buffer. The strongest inflammatory reaction was obtained by the removal of the roof of the other blister followed by the administration of autologous serum in the chamber. Cells in these blister fluids were recruited during 10 hours, of which the patients underwent hemodialysis for four hours, and thereafter collected for analysis. Similar techniques have been used in other groups of patients with other objectives [13–17].

In the present investigation, there was no significant difference in CD11b expression on monocytes in interstitial fluid aspirated from the blisters at 0 hours between healthy subjects and patients. After 10 hours, however, monocytes in the control blister from hemodialysis patients had significantly lower cell surface expression of CD11b as compared with cells collected in the corresponding blister of healthy subjects. The reason for this observation is not fully known, but may be a consequence of increased intravascular activation, due to generation of anaphylatoxins and inflammatory cytokines, leading to increased refractoriness to chemotactic factors at the site of inflammation in the interstitium. In an attempt to evaluate whether a low cell surface expression of CD11b on extravasated monocytes is a consequence of low levels of chemotactic factors in the blister fluid, monocytes from healthy blood donors were incubated in blister fluid collected from the active and control chambers from hemodialysis patients and healthy subjects. The concentration of CD11b-mobilizing factors was similar in the pools of blister fluid obtained from patients and healthy subjects. Thus, despite the fact that monocytes from hemodialysis patients migrate into an interstitial fluid containing equal biological activity of CD11b-mobilizing substances and the fact that the systemic chemotactic activity in terms of MCP-1 levels are increased, they have mobilized the receptor to a significant lesser extent.

In the chamber containing autologous serum (intense inflammation), the cell surface expression of CD11b on monocytes obtained from patients increased and reached levels obtained in the corresponding blister fluid of healthy individuals. Together, these observations favor the view that monocytes from dialysis patients have the capacity to mobilize CD11b to the same extent as cells from healthy individuals at the inflammatory spot, but that a more intense stimulus is required for such actions, probably because of a transient refractoriness. Further-

more, the number of monocytes at the site of inflammation in the interstitium in patients correlated to the expression of CD11b on transmigrating cells. It must be emphasized that during hemodialysis, a notable variation in blood leukocyte counts occurs because of sequestration and rebound phenomenon. The impact of this on the accumulation of monocytes rate must also be considered.

Leukocytes play an essential role as a first-line defense against invading microorganisms. A prerequisite for this action in inflammation is their capability to adhere to endothelial cells, extravasate, and subsequently transmigrate into the surrounding interstitial tissue. Monocytes originate from the myeloid line of differentiating cells in the bone marrow. Circulating monocytes change their phenotype after extravasation and differentiate into tissue-specific macrophages. Monocytes and macrophages therefore are proposed to play a critical role in the initiation and maintenance of inflammation and in wound healing. Most studies on granulae content in phagocytes have been conducted on neutrophils, and based on these studies, a hierarchy in mobilization of secretory vesicles, gelatinase granules, specific granules, and azurophil granules has been demonstrated [18, 19]. The granulae characteristics in monocytes are less well known, but the currently held concept is that monocytes contain three populations of granules, peroxidase positive, peroxidase negative, and secretory vesicles, the latter containing CD11b molecules [20]. Differences between monocytes and neutrophils with regard to the intracellular pool and location of adhesion molecules, as well as the sensitivity towards chemoattractants, have been reported [21].

During the leukocyte transmigration process, a shift in adhesion molecule phenotype generally occurs, which includes an up-regulation of CD11b in parallel with a down-regulation of L-selectin [15]. This alteration does also occur in monocytes, even if the magnitude of the shift can be less marked than in neutrophils [22]. This is in line with previously reported data in which we observed a less marked hemodialysis-induced shift in adhesion molecule phenotype in circulating monocytes than in neutrophils [6–8]. By comparing neutrophils accumulated in vivo in skin chambers to cells incubated under the same conditions in plasma filled skin chambers, Sengeloev et al demonstrated that granule exocytosis takes place during the transmigration and not during the stay in the collection chamber [15]. Furthermore, stimulation with fMLP resulted in an additional release of granules that indicates that transmigrating leukocytes can retain their ability to respond. Our data suggest that this ability is also valid for monocytes, which in the skin chamber with an intermediate inflammatory reaction had only partially up-regulated CD11b. In the skin chamber with an intense inflammatory reaction, however, the difference between patients and healthy subjects was no longer present.

Several specific chemokines may be involved in the effects observed on intravascular and extravasated monocytes at the different levels of inflammation in the present investigation. In a study by Kuhns, migration of leukocytes into the skin chamber fluid was detectable within 3 hours and appeared to plateau at 16 to 24 hours [14]. Several inflammatory mediators such as interferon-γ, complement fragment C5a, leukotriene B_4, IL-1, IL-6, IL-8, tumor necrosis factor, and granulocyte-macrophage colony-stimulating factor were detected at different time points [14]. Herein, we report increased levels of serum MCP-1 in hemodialysis patients and a correlation between MCP-1 levels and monocyte accumulation at an inflammatory site. This implies that MCP-1, a well-characterized monocyte-specific chemokine, contributes to monocyte activation and accumulation in hemodialysis patients [23]. One may speculate that the higher systemic concentration of MCP-1 in hemodialysis patients as compared with in healthy subjects may be necessary to maintain monocyte recruitment and thus contribute to the observed similar accumulation rate of monocytes at the site of inflammation in patients and healthy subjects. Other investigators have previously reported increased levels of the neutrophil specific chemokine IL-8 in dialysis patients [24], which supports a role for chemokines in the recruitment of leukocytes in hemodialysis patients.

The finding that inflammatory cells in patients on hemodialysis accumulate at an inflammatory site to the same degree as in healthy subjects but only partially up-regulate their CD11b/CD18 expression may have important clinical implications. Leukocytes with cytotoxic actions have to adhere to their targets cells (for example, virus-infected cells), mainly through CD11b/CD18 molecules, to kill them. CD11/CD18 also participates in phagocytosis [reviewed in 25–27]. Thus, the reduced capacity of transmigrating monocytes collected in the interstitium from patients on hemodialysis to mobilize CD11b/CD18 in response to intermediate inflammatory stimuli may result in a less effective host defense with regard to cell adhesion and phagocytosis of microorganisms.

In conclusion, monocytes that extravasate and transmigrate into inflammatory foci of hemodialysis patients during a 10-hour period, of which the four first hours were with Cuprophan dialysis treatment, have only partially mobilized CD11b in response to an intermediate inflammatory stimuli as compared with cells collected from corresponding skin blisters of healthy individuals, despite similar local biological activity of CD11b-mobilizing factors and increased systemic chemotactic activity.

ACKNOWLEDGMENTS

This study was supported by grants from the Karolinska Institute, the Swedish National Federation of Kidney Patients, the Swedish Society of Nephrology, The Lisa & Johan Grönbergs Stiftelse, Terumo Europe, and Fresenius AG, Germany. The authors want to thank Anette Bygdén and Titti Nieminen for skillful technical assistance.

Reprint requests to Dr. Pia Thylén, M.D., Ph.D., Department of Nephrology, Karolinska Hospital, S-171 76 Stockholm, Sweden. E-mail:pia@divmed.ks.se

REFERENCES

1. Adams DH, Lloyd AR: Chemokines: Leukocyte recruitment and activation cytokines. *Lancet* 349:490–495, 1997
2. Huber AR, Kunkel SL, Todd RF III, Weiss SJ: Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science* 254:99–102, 1991
3. Baggliolini M, Dewald B, Moser B: Human chemokines: An update. *Annu Rev Immunol* 15:675–705, 1997
4. Cheung AK, Parker CJ, Wilcox L, Janatova J: Activation of the alternative pathway of complement by cellulosic hemodialysis membranes. *Kidney Int* 36:257–265, 1989
5. Himmelfarb J, Zaoui P, Hakim R: Modulation of granulocyte LAM-1 and Mac-1 during dialysis: A prospective, randomized controlled trial. *Kidney Int* 41:388–395, 1992
6. Thylén P, Fernvik E, Lundahl J, Hed J, Jacobson SH: Cell surface receptor modulation on monocytes and granulocytes during clinical and experimental hemodialysis. *Am J Nephrol* 15:392–400, 1995
7. Thylén P, Fernvik E, Haegerstrand A, Lundahl J, Jacobson SH: Dialysis-induced serum factors inhibit adherence of monocytes and granulocytes to adult human endothelial cells. *Am J Kidney Dis* 29:78–85, 1997
8. Thylén P, Fernvik E, Lundahl J, Lins L-E, Jacobson SH: Monocyte and granulocyte CD11b/CD18 expression and sICAM-1 concentration in the interdialytic period. *Nephron* 74:275–282, 1996
9. Tielemans C, Husson C, Schurmans T, Gastaldello K, Madhoun P, Delville JP, Marchant A, Goldman M, Vanherweghem JL: Effects of ultrapure and non-sterile dialysate on the inflammatory response during hemodialysis. *Kidney Int* 49:236–243, 1996
10. Hakim RM, Breillatt JMLJ, Port FK: Complement activation and hypersensitivity reactions to dialysis membranes. *N Engl J Med* 311:878–882, 1984
11. Alvarez V, Pulido R, Campanero MR, Paraiso V, Landazur D, Sanchez-Madrid F: Differentially regulated cell surface expression of leukocyte adhesion receptors on neutrophils. *Kidney Int* 40:899–905, 1991
12. Skroeder NR, Kjellstrand P, Holmquist B, Kjellstrand CM, Jacobson SH: On complement net generation in fast hemodialysis: Are high blood flow rates bioincompatible? *Am J Kidney Dis* 25:896–903, 1995
13. Scheja A, Forsgren A: A skin chamber technique for leukocyte migration studies: Description and reproducibility. *Acta Path Microbiol Immunol Scand [C]* 93:25–30, 1985
14. Kuhns DB, Decarlo E, Hawk DM, Gallin JI: Dynamics of the cellular and humoral components of the inflammatory response elicited in skin blisters in humans. *J Clin Invest* 89:1734–1740, 1992
15. Sengeloev H, Follin P, Kjeldsen L, Lollike K, Dahlgren C, Boerregaard N: Mobilization of granules and secretory vesicles during in vivo exudation of human neutrophils. *J Immunol* 154:4157–4165, 1995
16. Yamada S, Mayadas TN, Yuan F, Wagner DD, Hynes RO, Melder RJ, Jain RK: Rolling in P-selectin-deficient mice is reduced but not eliminated in the dorsal skin. *Blood* 86:3487–3492, 1995
17. Raud J, Halldén G, Roquet A, van Hage-Hamsten M, Alkner U, Dahlén SE, Hedqvist P, Groenneberg R: Anti-IgE-induced accumulation of leukocytes, mediators, and albumin in skin chamber fluid from healthy and atopic subjects. *J Allergy Clin Immunol* 97:1151–1163, 1996
18. Borregaard N, Kjeldsen L, Rygaard K, Bastholm L, Nielsen MH, Sengeloev H, Bjerrum OW, Johnsen AH: Stimulus-dependent secretion of plasma proteins from human neutrophils. *J Clin Invest* 90:86–96, 1992
19. Kjeldsen L, Bainton DF, Sengeloev H, Borregaard N: Structural and functional heterogeneity among peroxidase-negative granules in human neutrophils: Identification of a distinct gelatinase-containing granule subset by combined immunocytochemistry and subcellular fractionation. *Blood* 82:3183–3191, 1993
20. Calafat J, Kuijpers TW, Janssen H, Borregaard N, Verhoeven AJ, Roos D: Evidence for small intracellular vesicles in human blood phagocytes containing cytochrome b558, and the adhesion molecule CD11b/CD18. *Blood* 81:3122–3129, 1993
21. Miller LJ, Bainton DF, Borregaard N, Springer TA: Stimulated mobilization of monocyte Mac-1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. *J Clin Invest* 80:535–544, 1987
22. Lundahl J, Hed J: Differences in the altered expression of L-selectin and Mac-1 in monocytes and neutrophils. *Inflammation* 18:67–76, 1994
23. Akahoshi T, Kobayashi N, Hosaka S, Sekiyama N, Wada C, Kondo H: In-vivo induction of monocyte chemotactic and activating factor in patients with chronic renal failure. *Nephrol Dial Transplant* 10:2244–2249, 1995
24. Niwa T, Miyazaki T, Sato M, Kambe F, Tsuzuki T, Uema K, Maeda K, Seo H: Interleukin 8 and biocompatibility of dialysis membranes. *Am J Nephrol* 15:181–185, 1995
25. Springer TA: Adhesion receptors of the immune system. *Nature* 346:425–434, 1990
26. Patarroyo M, Prieto J, Rincon J, Timonen T, Lundberg C, Lindholm L, Aasjoe B, Gahmberg CG: Leukocyte-cell adhesion: A molecular process fundamental in leukocyte physiology. *Immunol Rev* 114:67–108, 1990
27. Arnaout MA: Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood* 75:1037–1050, 1990